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COMPARISON OF THE SELECTIVE EFFECT OF TWO MUSTARD OILS AND THEIR GLUCOSIDES TO PIERIS LARVAE

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The effectiveness of various mustard oils (isothiocyanates) on attracting larvae of *Pieris rapae* has been shown previously (Hovanitz, Chang and Honch, 1963b, and Hovanitz and Chang, 1963a). In this paper, some data are presented to compare the effectiveness of two of these mustard oils with their corresponding glucosides. The combinations of mustard oils and glucosides being considered are allyl isothiocyanate with sinigrin, and benzyl isothiocyanate with glucotropaeolin. The naturally known occurrence of the substances in plants is indicated in Table 1 of the before mentioned paper (Hov., Chang and Honch, 1963b).

THE STRAINS AND EXPERIMENTAL SET-UP

The two strains of *Pieris rapae* used in these experiments are the same as used in previous experiments. The first, the kale strain, has been maintained ten or more generations on kale and the second, the mustard strain, has been maintained for more than ten generations on mustard.

The experimental set-up for these tests has been the same as for those tests previously made (Hovanitz and Chang, 1963a).

ATTRACTION OF GLUCOSIDES

The glucoside, glucotropaelum, is known to occur naturally in a wide variety of plants, some natural food plants of *Pieris rapae* and others not.

Previously published results of tests on the mustard oils corresponding to these glucosides have indicated that larvae of *Pieris rapae* bred on mustard have no more selection for benzyl isothiocyanate than for distilled water, where the dilutions have

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TABLE 1. Attraction of glucosides of various concentrations to Pieris rapae larvae

	Test	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	Water	None	Total
glucoside: glucotropaeolum larvae: mustard-strain	1			34 11.33	37 12.33	61 20.33	49 16.33	56 18.67	24 8.00	39 13.00	300
	2			66 20.63	44 13.75	51 15.94	51 15.94	49 15.31	34 10.62	25 7.81	320
	3			38 15.83	37 15.41	45 18.75	37 15.42	33 13.75	32 13.33	18 7.50	240
	Total			138 16.05	118 13.75	157 18.25	137 15.93	138 16.04	90 10.46	82 9.53	860
glucoside: glucotropaeolum larvae: kale-strain	1			20 10.00	50 25.00	34 17.00	27 13.50	32 16.00	12 6.00	25 12.50	200
	2			24 15.00	27 16.87	22 13.75	25 15.62	37 23.12	13 8.12	12 7.50	160
	3			36 22.50	28 17.50	22 13.75	24 15.00	13 8.12	14 8.75	23 14.37	160
	Total			80 15.38	105 20.19	78 15.00	76 14.62	82 15.76	39 7.50	60 11.53	520
glucoside: sinigrin larvae: mustard-strain	1			48 17.14	19 6.78	47 16.78	42 15.00	48 17.14	19 6.78	57 20.36	280
	2			38 11.87	54 16.87	52 16.25	63 19.69	47 14.69	40 12.50	26 8.12	320
	Total			86 14.33	73 12.17	99 16.50	105 17.50	95 15.83	59 9.83	83 13.83	600
	3	81 13.50	97 16.16	102 17.00	98 16.35	84 14.00			69 11.50	69 11.50	600
glucoside: sinigrin larvae: kale-strain	1			35 13.46	44 16.92	40 15.38	40 15.38	25 9.61	32 12.31	44 16.92	260
	2			14 10.00	23 16.43	34 24.28	26 18.57	18 12.86	12 8.57	13 9.28	140
	3			77 24.06	44 13.75	48 15.00	51 15.94	39 12.19	21 6.56	40 12.50	320
	Total			126 17.50	111 15.41	122 16.94	117 16.25	82 11.38	65 9.03	97 13.47	720

ranged from 10^{-5} to 10^{-9}. On the other hand, similar larvae tested for allyl isothiocyanate showed a strong selection for this mustard oil at a concentration of 10^{-6} (Hovanitz, Chang and Honch 1963b). Larvae bred on kale tested in the same way showed a decided preference for a more dilute concentration, 10^{-8} (Hovanitz and Chang 1963a).

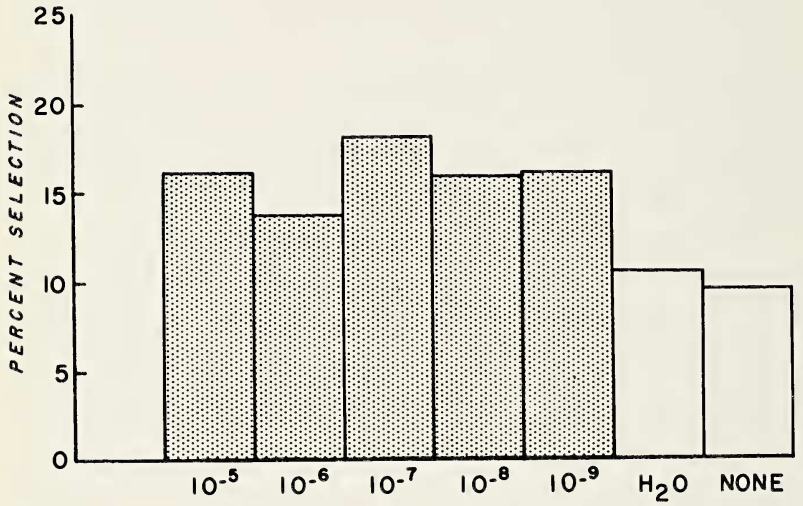
Tests of larvae bred on kale, and other tests of larvae bred on mustard, were made on the two glucosides (Table 1). These tests have indicated that both glucosides have some selective influence on the larvae but that the selection is not very much greater than that on water alone.

Glucotropaeolum tested with mustard-strain larvae at concentrations of 10^{-5} to 10^{-9} and distilled water showed an average selection of 18 percent for a concentration of 10^{-7} as compared with water at 10 percent (Fig. 1). The other concentrations were not much different at 13 to 16 percent. This compares with no apparent selection for the corresponding mustard oil, benzyl-isothiocyanate.

Glucotropaeolum, tested again with kale-strain larvae at the same concentrations, showed the highest rate of selection, 20 percent, at 10^{-6} as compared with water at 7.5 percent and other concentrations at 15 percent (Fig. 2). This indicates a positive selection for the glucoside, whereas no selection was indicated for the corresponding mustard oil, benzyl isothiocyanate.

Sinigrin, the glucoside of allyl-isothiocyanate, was tested with mustard-strain larvae at concentrations of 10^{-5} to 10^{-9} and distilled water in a series of two tests and at concentrations of 10^{-3} to 10^{-7} in a third. The average of the first two tests indicate a high selection of 17.5 percent at a concentration of 10^{-8} as compared with water at ten percent though other concentrations were not greatly different, at 12 to 16.5 percent (Fig. 3). It is possible that there is little significant effect of change of concentration.

The same tests were made over to see if a higher concentration of sinigrin had a greater influence on selection. Concentrations were arranged from 10^{-3} to 10^{-7} (Fig. 5). The highest selection, in this series, 17 percent, was at 10^{-5}, with other concentrations ranging from 13.5 to 16 percent, compared with water alone at 11.5 percent. There seems to be little difference in selection at the different concentrations except that water is selected less than the glucoside sinigrin. These results differ



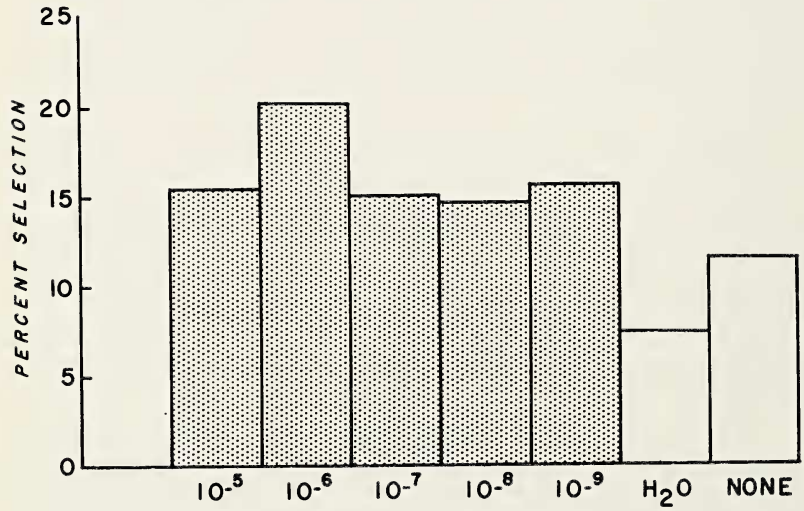


Fig. 1. The percent selections by larvae of *Pieris rapae* of a standard bred strain tested for attraction to various dilutions of glucotropaeolum.

Fig. 2. Similar selections by larvae from kale bred strains of *Pieris rapae* tested to glucotropaeolum.

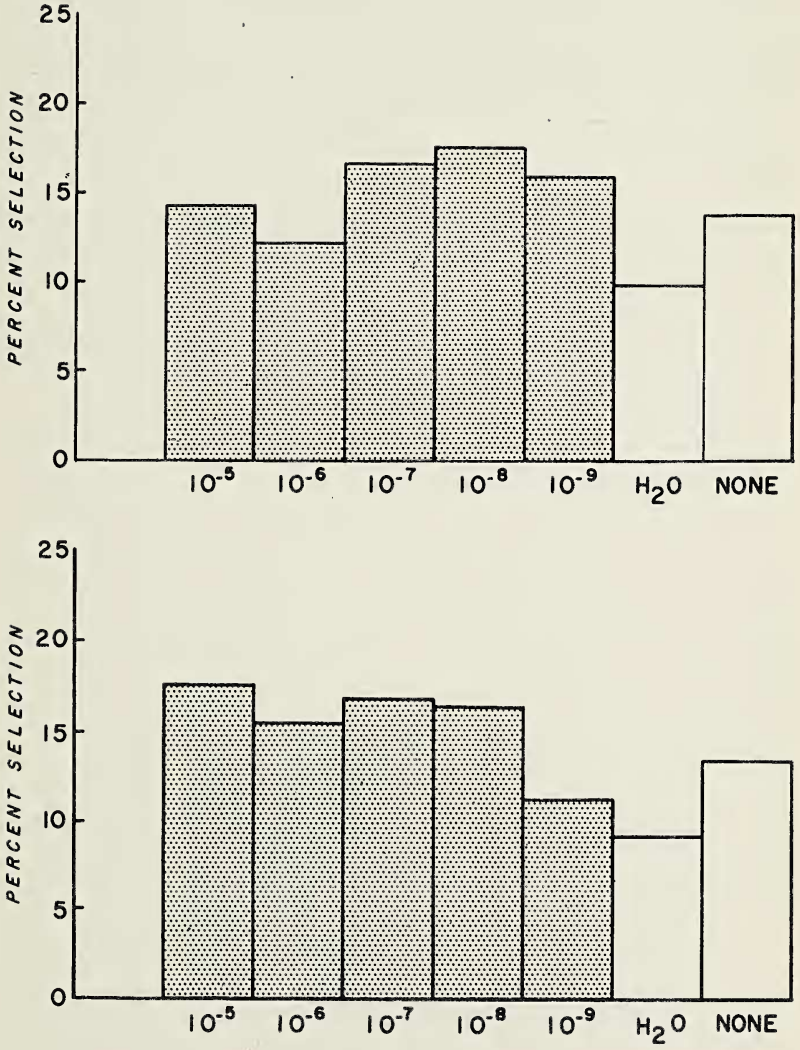


Fig. 3. The percent selection by larvae of mustard strain *Pieris rapae* tested for attraction to various dilutions of sinigrin.
Fig. 4. Similar selections by larvae of kale strain *Pieris rapae* to sinigrin.

markedly from the selection data of mustard-strain larvae for allyl-mustard oil, with a high selection of 30 percent at 10^{-6} (Hovanitz and Chang, 1963b).

Sinigrin was also tested with larvae from the kale strain (Fig. 4). The concentration of -0^{-5} led to selections of 17.5 percent followed closely by 17 percent at 10^{-7}. This corresponded to water at 9 percent, and a range of 11 to 16 percent for the other concentrations. These results also differ markedly from the selection data of kale strain larvae for allyl-mustard oil, with a high selection of 34 percent at 10^{-8} (Hovanitz and Chang 1963a).

CONCLUSIONS AND DISCUSSION

The results of these experiments lead to the following conclusions:

1. Glucotropaeolum is more effective in attracting larvae of *Pieris rapae* than its corresponding oil, benzyl-isothiocyanate. However, the oil has no apparent attractive influence at all and the glucoside has only a slight effect.

2. There is little difference in selection between the various concentrations of the glucoside.

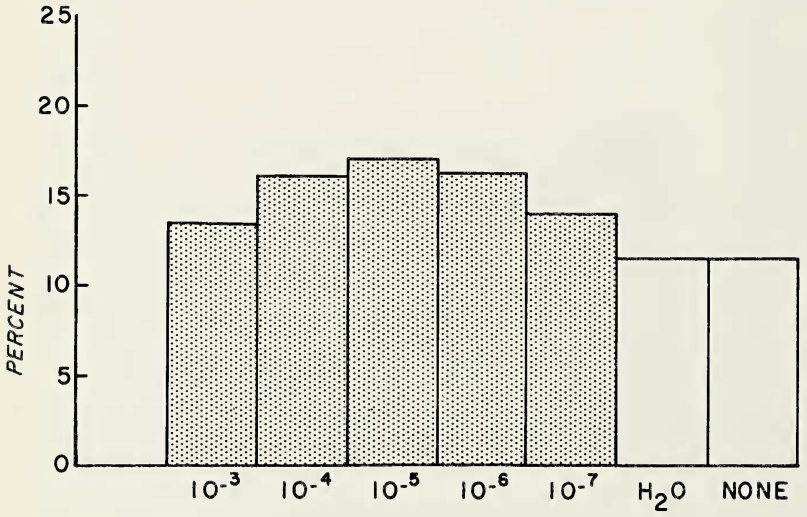


Fig. 5. The percent selections by larvae of mustard strain *Pieris rapae* tested for attraction to various dilutions of sinigrin.

3. There is little difference in selections made by the larvae toward glucotropaeolum depending on whether they were from the mustard strain or the kale strain, though it is possibly significant that a higher selection may occur toward a concentration of 10^{-7} for the mustard strain larvae as compared with a concentration of 10^{-6} for kale strain larvae.

4. Sinigrin is less effective in attracting larvae of *Pieris rapae* than its corresponding oil, allyl isothiocyanate.

5. There is little difference in selection between the various concentrations of the glucoside.

6. There is little difference in selections made by the larvae toward sinigrin depending on whether they were from the mustard strain or the kale strain. It is possibly significant that a higher selection may occur for a higer concentration of sinigrin for the mustard-strain larvae. This result is opposed to the selection data obtained for the same strains tested for allyl mustard oil (Hovanitz and Chang, 1963a).

Interpretation of these results may depend finally upon future experiments which would differentiate the ability of the larvae to perceive odors from a distance. The experimental set-up for these tests has been based upon the ability of the larvae to react to odors from a distance of over 100 mm. If the attraction of larvae to the glucoside sinigrin is really based upon attraction to the allyl mustard oil vapors emanating from sinigrin by hydrolysis, then there may be very little difference in the total amount of the mustard oil present at any time and this could conceiveably account for the almost lack of selective differentiation between various concentrations of sinigrin, unlike that of the mustard oil itself. If the mustard oil emanating from the sinigrin were restricted by the speed of the reaction of hydrolysis, then the oil vapors would possibly be dissipated quickly in the air and not carry far enough to attract the larvae differentially. On the other hand, selections made close at hand, within a few millimeters, might serve to bring about such a differentiation.

The lack of any clear-cut selective differences under the conditions of these experiments is strange in view of the great effectiveness of larval selections of different food plants from the same distances. It is possible that the plants have a means to store the mustard oils obtained by enzymatic hydrolysis of sinigrin in greater concentrations that is possible where the sinigrin is

directly exposed to the air. It is difficult experimentally to control the concentration of mustard oils for experimental purposes because of their great volatility.

LITERATURE CITED

HOVANITZ, W. and V. C. S. CHANG, 1963. a. Selection of allyl isothiocyanate by larvae of *Pieris rapae* and the inheritance of this trait. *J. Res. Lepid.* 1 (3): 169-182.

HOVANITZ, W., V. C. S. CHANG and G. HONCH, 1963. b. The effectiveness of different isothiocyanates on attracting larvae of *Pieris rapae*. *J. Res. Lepid.* 1 (4): 249-259.